Cellular radiation response of mouse embryonic stem cell derived cardiomyocytes *

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To examine putative adverse effects of ionizing radiation on electrophysiological properties of cardiomyocytes, we recently performed a series of experiments with mouse embryonic stem cell derived cardiomyocytes (mESC-CM). For these studies mESC-CM were generated via embryoid body (EB) formation. Yet, this system proved to be unsuitable due to a low number of cardiomyocytes formed and pronounced inter- and intra-experimental variations [1]. To overcome these limitations pure mESC-CM (Cor.At cardiomyocytes, Axiogenesis) have been used in subsequent electrophysiological studies [2]. As a complement we also examined the cellular radiation response of Cor.At cells. For the experiments cells were seeded in chamber slides. Two days after seeding cells were irradiated with 0.5, 1 or 2 Gy X-rays (250 kV, 16 mA, 1.5 Gy/min). Radiation effects were examined at 1, 2, 3 and 4 days after exposure. Apoptosis, micronucleus formation and binucleation were analyzed by means of a DAPI nuclear staining; while the expression of connexin 43 (Cx 43) and troponin T was measured by immunocytochemistry (Figure 2 B, C). Our experiments show a dose- and time-dependent increase in the number of cells with micronuclei and in the number of apoptotic cells. Exemplarily, in figure 1 the apoptotic response of control and irradiated cultures (2 Gy X-rays) is plotted. In the control the spontaneous frequency of apoptotic cells was $\leq$8%. At 1 day after exposure to 2 Gy X-rays the number of apoptotic cells was as high as in the control samples, but it increased to 17% and 33% at day 2 and 4 after exposure. Interestingly, the number of binucleated cells, indicating maturation, rose in all samples with time and was not affected in the dose range examined (data not shown). Additionally, we analyzed the expression of Cx 43 (Figure 2 C), a main compound of cardiac gap junctions, in comparison to the expression of troponin T (Figure 2 B), a structural cardiac protein. An up-regulation of Cx 43 expression after an exposure to carbon ions has been observed for rabbit cardiomyocytes resulting in improved conductivity [3]. The analysis performed so far revealed pronounced sample variations, i.e. no consistent picture emerged. Altogether, our first experiments show that doses up to 2 Gy X-rays do not affect the maturation of Cor.At cardiomyocytes. However, a dose-dependent increase in the number of apoptotic cells and cells carrying micronuclei was detected. Due to the high sample variations, no firm conclusions on the impact of X-rays on the expression of Cx 43 and subsequently cellular communication can be drawn. In further studies refined technique will be used to study the expression of specific cardiac genes and first experiments with high LET radiation will be performed.

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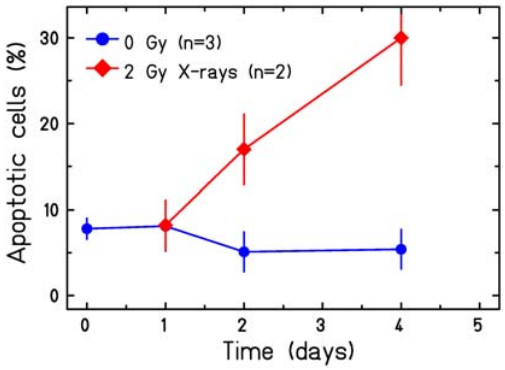

Figure 1: Percentage of apoptotic Cor.At cardiomyocytes in unirradiated or X-ray irradiated cultures. Cells were exposed at day 0.

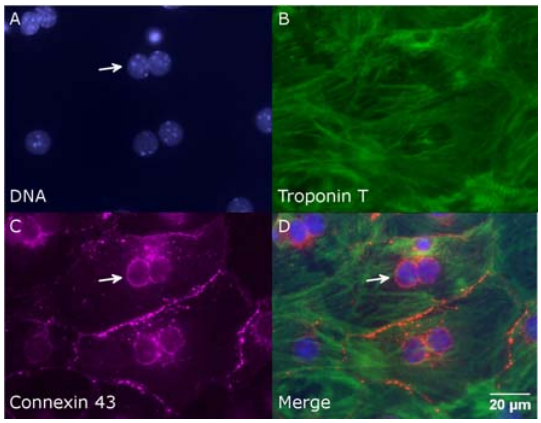

Figure 2: Immunofluorescence staining of Cor.At cells for connexin 43 (A) and troponin T (B), nuclei in (C) are stained with DAPI; merged images are shown in (D). Binucleated cells are indicated by an arrowhead in (C, D).

References

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